

CLAIMS

1. An assay for detecting an effect a compound has on a membrane receptor/reporter fusion protein, comprising the steps of:

a) adding the compound to a cell comprising said membrane receptor/reporter fusion protein; and

b) detecting any change of said receptor/reporter fusion protein.

2. The assay according to claim 1 wherein said assay is used to screen compounds for their effect on membrane receptors.

3. The assay according to claim 2 for screening compounds which modulate the activity of wild type and/or mutant membrane receptors.

4. The assay according to claim 3 wherein the membrane receptor is a wild type receptor and any change is detected as a decrease in activity of the receptor/reporter fusion protein.

5. The assay according to claim 3 wherein the membrane receptor is a constitutively active mutant receptor and any change is detected as an increase in activity of the receptor/reporter fusion protein.

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6. The assay according to any preceding claim wherein said assay is used to identify compounds that disrupt normal membrane receptor interactions, or can in themselves disrupt such interactions.

7. The assay according to any preceding claim for detecting a compound which serves as an inverse agonist, antagonist or agonist of the membrane receptor.

8. The assay according to claim 7 wherein said inverse agonist, antagonist or agonist of the membrane receptor is used in the study of receptor function or therapy.

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9. The assay according to any preceding claim wherein said membrane receptor is a growth factor receptor, cytokine receptor, ion channel, integrin, or G-protein receptor.

10. The assay according to claim 9 wherein said membrane receptor is a subtype, mutant, homolog, or chimeric form of a wild-type receptor.

11. The assay according to claim 10 wherein said mutant is a constitutively active mutant.

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12. The assay according to claim 11 wherein the constitutively active mutant receptor/reporter fusion protein is initially unstable, such that the reporter activity is detected at a basal level and wherein after binding of a compound to the receptor/reporter fusion protein is stabilised and an increase in reporter activity is observed.

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B3* 13. The assay according to any one of claims 9-12 wherein said G-protein coupled receptor is a dopamine receptor, a muscarinic cholinergic receptor, an α -adrenergic receptor, a β -adrenergic receptor, an opiate receptor, a cannabinoid receptor, a serotonin receptor or a protease activated receptor.

14. The assay according to any preceding claim wherein the receptor/reporter fusion protein is expressed from a nucleic acid construct comprising a gene encoding said reporter protein which is fused in-frame to the 5' or 3' end of a gene encoding said membrane receptor.

15. The assay according to any preceding claim wherein the functionality of said membrane receptor/reporter fusion protein is substantially unaffected by fusion of the reporter protein to the receptor.

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13 16. The assay according to any preceding claim wherein said reporter protein is Green Fluorescent Protein (GFP), or active variant thereof.

17. The assay according to claim 16 wherein light emitted by said GFP protein is detected by fluoumetry, FACS, or microscopy techniques.

18. The assay according to any one of claims 1-15 wherein said reporter protein is *Renilla reniformis* (sea pansy) luciferase protein, secreted placental alkaline phosphatase (SEAP), β -lactamase, galactosidase, firefly (*Photinus pyralis*) luciferase, blue fluorescent protein, yellow fluorescent protein, cyan fluorescent protein.

19. The assay according to claim 18 wherein said reporter protein is luciferase which is detected in a microplate luminometer or using a CCD imaging system.

14 20. The assay according to any preceding claim wherein said reporter protein is used to localise and/or quantify the membrane receptor.

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21. An assay according to any preceding claim wherein any change of said membrane receptor/reporter fusion protein is detected as a change in cellular localisation of the receptor/reporter fusion protein, or semi-quantitatively by the synthesis or degradation of said receptor/reporter fusion protein.

22. An assay according to any preceding claim wherein said detection of any change of said membrane receptor/reporter fusion protein is carried out with cells placed on the surface of a microscope slide or the like.

23. The assay according to any preceding claim wherein said detection of any change of said membrane receptor/reporter fusion protein is carried out on cells placed in a well of a microtitre plate or the like, such as a 96-well plate.

24. An assay for detecting a compound which has an effect on a membrane receptor, comprising the steps of

a) expressing a membrane receptor/reporter fusion protein in a cell;

b) detecting a basal level of reporter activity;

c) adding a test compound to the cell; and

d) detecting a resulting activity of the reporter protein, wherein alteration of reporter activity with respect to the basal level is due to the test compound having an effect on the membrane complex.

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25. The assay according to claim 24 wherein the membrane receptor is a wild type receptor and alteration is a decrease in reporter activity.

26. The assay according to claim 24 wherein the membrane receptor is a constitutively active mutant receptor and alteration is an increase in reporter activity.

27. A membrane receptor/reporter fusion protein comprising a constitutively active mutant receptor which has a reporter added in-frame at the C-terminal.

28. The membrane receptor/reporter fusion protein according to claim 27 wherein the constitutively active mutant receptor is a GPCR.

29. The membrane receptor/reporter fusion protein according to either of claims 27 or 28 wherein the reporter protein is GFP or luciferase.

30. A compound identified by the assay according to any one of claims 1 - 26.

31. Use of a compound identified by the assay according to any one of claims 1 - 26 in therapy.

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II
could be used
in vitro

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